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THE PATHOGENICITY OF *B. MELITENSIS* AND *B. ABORTUS* FOR GUINEA-PIGS

STUDIES ON THE GENUS *BRUCELLA* NOV. GEN. IV

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In a preliminary communication¹ it was demonstrated that 5 old stock strains of *B. melitensis* inoculated by the intratesticular route produced in guinea-pigs anatomic lesions, which could not be distinguished from those seen in this species infected with *B. abortus*. In fact, the pathogenicity tests furnished additional evidence to support the contention of recent workers that *B. melitensis* and *B. abortus* are closely related. It was shown that the etiologic agent of undulant fever of man persists for at least 2 months in the tissues of infected animals and exhibits a peculiar affinity for the spleen, lymphnodes, liver and sex organs. Comparative serologic studies with the serums of the infected animals and the cultures obtained from their organs conclusively disproved any assumption that the infections might have been caused by accidentally ingested abortion bacilli, instead of by the inoculated *B. melitensis*. In order to verify the preliminary observations, a total of 22 strains of *B. melitensis*, obtained from English, Algerian and Italian laboratories, was subsequently tested on guinea-pigs. Some of the findings necessitated a comparative study of the lesions produced by *B. abortus*. It has therefore been considered advisable to include in this paper data on the experimental guinea-pig disease produced by the bacteria of the *Brucella* group, which have been collected in this laboratory during the last 6 years.

METHODS

The cultures used to inoculate the guinea-pigs were grown on glycerol peptic digest agar (P_H 6.8-7.2); only the tubes seeded with *B. abortus* cultures were sealed. The injections were made either into the abdominal cavity or into the parenchyma of the testicle. The latter mode of infection was carried out as follows: The anterior limbs and the left leg of the guinea-pig were held by an assistant,

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¹ Proc. Soc. Exper. Biol. and Med., 1919, 16, p. 757.

and the animal was placed on its back. The operator fixed with the left hand the right or left testicle in the scrotum; occasionally gentle pressure on the abdominal wall close to the pelvic ring was necessary to bring the organ into proper position. The skin was thoroughly cleansed with alcohol and ether and then painted with iodine, or better, with a mixture of brilliant green and crystal violet in 50% alcohol; a fine needle was inserted through the scrotum into the parenchyma and, as a rule, 0.2-0.5 c c of the bacterial suspension was injected. The fingers of the left hand controlled the location of the needle and the intra-testicular tension; particular care was exercised to prevent a rupture of the punctured testicle by the rapid injection of excessive amounts of the inoculum. The needle puncture was closed with iodized collodium.

The course of the infection was followed by regular weight determinations and skin tests. The preparation of the antigens, the method of application and the interpretation of the cutaneous reactions have been stated in the previous papers.² When the animals were considered ready for bacteriologic examinations, they were chloroformed, necropsied and the lesions noted; portions of the tissues were preserved for sections, and cultures were prepared from the spleen, liver, kidneys, lymphnodes, bonemarrow, urine, bile, abscesses and bone lesions. Sheep-blood-peptic digest agar plates and peptic digest agar slanted were the mediums employed. Pieces of tissue of guinea-pigs infected with *B. abortus* were rubbed over the entire surface of the agar slant, while sections of organs of the animals inoculated with *B. melitensis* were spread over blood plates. The tubes were sealed with paraffin. The colonies were identified by microscopic examination and by carefully conducted agglutination tests. Specific anti-serums unabsorbed and absorbed with different strains were continuously used, and the procedures were followed, as outlined in paper II.³ Complete agglutination tests were also carried out with the serum of the sacrificed guinea-pigs. It is evident that the existence of an infection in the guinea-pigs was considered proved only by the presence of (1) positive skin reactions; (2) distinct anatomic changes in the spleen, the liver, the lymphnodes, the testes, etc.; (3) microscopic changes in the form of focal lesions consisting of nests of actively growing epithelioid cells and connective tissue hyperplasia; and (4) positive serum reactions, particularly agglutination reactions in dilution

² Amer. Jour. Dis. Child., 1918, 36, p. 268; 1919, 38, p. 577.

³ Feusier and Meyer: Jour. Infect. Dis., 1920, 27, p. 185.

above 1:100.⁴ The nature of the etiologic agent responsible for the above lesions and reactions, whether *B. abortus* or *B. melitensis*, was decided by (5) isolation of the bacteria from the tissues; (6) identification of the organisms by agglutination tests with serums specific for the various groups of the genus *Brucella*; (7) by absorbing the serum procured from the infected guinea-pigs with various antigens; and (8) by "passage" experiments. In the latter tests, either cultures obtained from the tissues on the first generation or the emulsions of portions of the organs were inoculated into guinea-pigs. Previous tests have demonstrated that *B. abortus* infections are always successfully perpetuated in this manner and that, as a rule, the anatomic lesions become more striking in the course of these "passages."

Early in the course of the experiments, the observations of Surface⁵ on spontaneous *B. abortus* infections among guinea-pigs under crowded laboratory conditions were recalled and caused a feeling of uncertainty. In spite of the fact that such spontaneous epizootics, due to organisms of the *Brucella* group, had not been encountered among the many thousands of guinea-pigs observed during the last six years in our laboratory, the laborious and time-consuming application of all the methods mentioned was considered necessary in order to avoid even the suspicion of possible spontaneous infection. Eliminating all conceivable sources of error, the data which will be presented conclusively demonstrate that *B. melitensis* can provoke in a certain percentage of guinea-pigs an infection indistinguishable from abortion disease.

EXPERIMENTS WITH *B. MELITENSIS*

Most of the publications on undulant fever convey the impression that attempts to excite a lethal infection in the usual laboratory rodents are for the most part unsuccessful, and in order to produce a fatal disease it is necessary to introduce enormous numbers of organisms subcutaneously or intraperitoneally. It is said that the infection follows a protracted course, and weeks, even months, may pass before death occurs. A careful review shows that at least for the guinea-pig certain definite lesions have been noted by a number of observers.

Durham,⁶ in 1898, by intraperitoneal injections, produced in guinea-pigs a number of infections which were chronic in character. He demonstrated *B. melitensis* in the urine and in the intensely engorged kidneys of these animals, which died or were killed long periods (74-94 days-7 months) after inoculation.

⁴ Repeated cutaneous injections of bacterial proteins may cause the appearance of agglutinins.

⁵ Jour. Infect. Dis., 1912, 11, p. 464.

⁶ Jour. Pathol. & Bacteriol., 1899, 5, p. 377.

In two instances, the spleen was much enlarged, but in the others the organ showed nothing abnormal; however, the animals were invariably emaciated. The serum of the inoculated guinea-pigs agglutinated *B. melitensis* in high dilutions (for example, 51st day, 1:200; 74th day, 1:1000). The same worker also injected guinea-pigs by the subdural route with cultures whose virulence had been increased by previous intracerebral passage, a method which had originally been used by Cantani to raise the virulence of *B. influenzae*. The course of the infection was acute, and in the animals that died the organisms were isolated from the viscera.

In 1904, Carbone,⁷ who later died of undulant fever contracted in his laboratory, showed that male guinea-pigs often developed a purulent inflammation of the tunica vaginalis, following intraperitoneal injection of *B. melitensis*.

It is to Eyre,⁸ however, that most credit is due for having studied the *B. melitensis* in relation to guinea-pig infections. He showed that following the first isolation from human cases, the *B. melitensis* could kill guinea-pigs only when given subcutaneously, intravenously or intraperitoneally in tremendous doses. Infection was also possible by intracranial inoculation. Using a freshly isolated strain, the infection was chronic, the animals living from 100 to 200 days. By repeated intracranial injections, he was able to increase the virulence of the organisms, so that the pigs died within 24 to 72 hours after an intravenous injection, 3 to 12 days after an intraperitoneal inoculation and 9 to 36 days after an intracranial inoculation.

Eyre described two types of infection in guinea-pigs, the acute and chronic, as follows:

Acute Melitensis Infection.—"Several days after a moderate dose of a highly virulent culture, or a heavy dose of a less virulent culture, the animal dies. For from two to three hours after the injection, he seems to be all right and eats well, but loss of weight sets in, which is highly characteristic for the disease. At first, the temperature drops rapidly, then rises. The animal sits huddled up, will not eat, becomes weaker and, if disturbed, goes into tonic convulsions. This stage of increased irritability goes gradually into a stage of coma, from which the animal can be aroused with difficulty, and in which he remains about 24 hours with a subnormal temperature. Sometimes he has a convulsion before death, but usually it occurs without warning. The postmortem findings are those of acute septicemia. The organisms can be recovered from all the tissues and organs. In male animals, following intraperitoneal infections, there develops a bilateral purulent process in the tunica vaginalis with beginning hypertrophy of the testes. This pus contains numerous organisms."

Chronic Melitensis Infection.—"With small doses of virulent, or fair doses of moderately virulent organisms, the animal gets a chronic infection. Aside from a progressive loss of weight and a severe anemia, there are no characteristic symptoms. Even the temperature is not much changed. The incubation period of the chronic variety of the disease is from two to three days. Then comes a time, three to six days, when the animal is perceptibly sick. He will not eat, stays in a corner of his cage, loses weight and is very weak. He gradually improves, eats well, even ravenously, and although losing no more weight, he never regains his original weight. Following weeks or months, during which, aside from the loss of weight, the animal seems well, he suddenly dies. Post-mortem cultures from the liver are sterile; from the spleen and bonemarrow there is sometimes a scanty growth, although these are also often sterile. From the kidneys and the urine a good growth of *B. melitensis* is obtained."

⁷ Arch. per le sc. med., 1904, 28, p. 273.

⁸ Report Mediterranean Fever Commission, 1905, Part 2, p. 67.

Nicolle and Conseil,⁹ in 1909, reported on 2 guinea-pigs, which had naturally acquired a *B. melitensis* infection in a stable, where infected maltese goats had been kept. The serum of both animals agglutinated *B. melitensis* in a dilution of 1:300 and the specific organism was isolated from the spleen and liver of one guinea-pig. The blood, urine and bile were sterile. In a subsequent paper¹⁰ these two investigators recorded some inoculation experiments on guinea-pigs with a strain recently isolated from the milk of an infected goat and they stated that "Contrairement a l'opinion classique, nos expériences ont donné constatement un resultat positif; le cobaye est donc un animal parfaitement sensible au *M. melitensis*." One guinea-pig, which had been injected with the growth of one ¼ agar slant and sacrificed on the 138th day, exhibited a slight hypertrophy and a granular appearance of the spleen. The same findings were made on a similarly treated guinea-pig, which was killed on the 42nd day. Feeding of cultures to 2 guinea-pigs for 10 consecutive days produced the same lesions. Spleen cultures were always positive, while *B. melitensis* was also isolated from the urine of 2 animals. The serum agglutinated the inoculated organism in dilutions of from 1:250 to 1:1000.

Sangiorgi (1913)¹¹ inoculated 3 guinea-pigs intraperitoneally with the blood of an undulant fever patient. The blood of 2 of the animals, when killed on the 30th day, agglutinated *B. melitensis* in a dilution of 1:100, and the spleens were greatly enlarged and soft ("la milza era molto in grandita, friabile"). In a recent study by Tallo,¹² it is shown that *B. melitensis* may persist for at least 35 days in the gallbladder and bile of guinea-pigs, which have been injected intravenously. These observations confirm those of Eyre and others and show that *B. melitensis* may leave the body by way of the alimentary canal, in the intestinal mucus and in the feces.

It is evident, from this review, that guinea-pigs are susceptible to *B. melitensis* and that a chronic disease with an enlargement of the spleen and a persistence of the inoculated bacteria in the tissues and secretions has been repeatedly noted. The significance of these facts has, however, not been appreciated until a study of *B. melitensis* by guinea-pig inoculations. Preliminary experiments,¹ conducted by the writers, resulted in negative postmortem and cultural findings until a special mode of injection, namely, the intratesticular route, was chosen. Some observations, which will be detailed in a subsequent chapter, had indicated that *B. abortus* possessed a striking affinity for the sex organs. As the two bacteria, *B. abortus* and *B. melitensis*, were morphologically, biochemically and serologically closely related, it was suspected that the sexotropic pathogenicity of *B. abortus* might also be characteristic of the organism of undulant fever and the possibility, therefore, of producing specific lesions by injections of the virus into the testes suggested itself. The majority of the inoculations reported in the following were

⁹ Compt. rend. Soc. de biol., 1909, 66, p. 503.

¹⁰ Ibid., 1909, 67, p. 267.

¹¹ Pathologica, 1913, 5, p. 554.

¹² Ibid., 1919, 11, p. 401.

made intratesticularly. Successful infections have, however, also been noted following intraperitoneal injections. The data dealing with the observations on 203 guinea-pigs are also presented in the following.

EXPERIMENTS WITH *B. MELITENSIS* STRAINS 1, 2, 3, 4, 5, 7,
8, 9, 10, 23, 24 and 25¹³

One tenth slant of a glycerol peptic digest agar culture was inoculated into the right testicle of guinea-pigs; as a rule, a series of 4 to 6 animals were infected. A total of 60 guinea-pigs was used; they all survived the infection and were sacrificed at different times after the inoculation, the period varying from 6 weeks to 6 months. Repeated skin tests gave negative reactions. At necropsy, the tissues were free from lesions, and the serum failed to agglutinate the strain of *B. melitensis* used for the inoculations in dilutions greater than 1:40.

EXPERIMENTS WITH A POLYHOMOGENOUS MIXTURE OF *B. MELITENSIS*
STRAINS 2, 3, 4, 5, AND 11

The results on the first series of 8 guinea-pigs have already been reported in a previous communication.¹ The cultures obtained from the tissues of 2 guinea-pigs (614 and 615) which exhibited typical necropsy findings were used for further pathogenicity tests. The results are tabulated below.

MELITENSIS CULTURE FROM URINE AND TESTIS OF GUINEA-PIG 614
INOCULATED INTRATESTICULARLY

Guinea-Pig 654.—Sacrificed on 119th day; skin tests always positive; necropsy revealed small spleen, but typical lesions in lymphnodes and liver.

Guinea-Pig 655.—Died on 45th day with typical lesions.

Spleen emulsion inoculated with negative results intratesticularly into four (709, 710, 711, 712) and intraperitoneally into two guinea-pigs (713, 714).

Spleen culture, 2d generation, was identified in Monkey 15 as a *B. melitensis*. One guinea-pig (733), of 8 inoculated, revealed a typical lesion at necropsy.

The histories of guinea-pigs 655 and 733 are presented in detail.

Guinea-Pig 655.—Weighing 540 gm., was inoculated into the right testicle with 1/20 slant of a young culture (2d generation) of *B. melitensis* isolated from the testis and urine of guinea-pig 614. On the 44th day after the inoculation (weight 537 gm.) the animal was subjected to a skin test with "aborto" and "melitensis protein." The following day, the guinea-pig exhibited marked skin reactions, coryza and dyspnea; it apparently died from anaphylaxis 21 hours after the cutaneous injection of the bacterial proteins. Necropsy demonstrated

¹³ Histories of Strains: 1: marked R. A. M. C.; 2: U. S. Navy; 3: stock 22; 4: Dr. Arneth; 5: stock; all obtained from the Hygienic Laboratory, U. S. Public Health Service 1918; 7: marked R. Mum. 5; 8: marked R. Monaco; 9: R B₂ paramelitensis, obtained from Dr. E. Sergent, Institut Pasteur d'Algerie, 1919; 10: obtained from the Institute for Infectious Diseases, Bern, 1914; 23: paramelitensis R. A. M. C., obtained from the Lister Institute; 24: marked Celli obtained from Dr. A. Ascoli, Modena, Italy; 25: marked "Douglas" obtained from Dr. J. W. H. Eyre, Guy's Hospital, London, 1919.

an enlarged, nodular and typical spleen (weight 1.9 gm.), large and firm lymphnodes, numerous foci in the liver and bilateral atrophy of the testes. The microscopic findings were characteristic. *B. melitensis* was isolated from the spleen and the left precrucial lymphnodes; the urine, bile, bonemarrow, liver and testes were sterile. The serum gave the following agglutination reactions:

B. melitensis 1, 2, 3, 4, 5, 8, 11 — 1:400 = 1:1000 depending on the antigen; after absorption with *B. abortus* 80, 1:200.

B. melitensis 7 and 9 — <1:40.

B. abortus 80 and 320 — 1:100; after absorption with *B. abortus*, <1:40.

Guinea-Pig 733.—Weighing 450 gm., was inoculated intratesticularly with one 1/10 slant of a culture (1st generation) obtained from the spleen of guinea-pig 655. The skin reactions were positive. On the 155th day, the animal weighed 221 gm. and was chloroformed. The necropsy findings were typical, the spleen weighed 1.4 gm., and the liver was studded with small, grayish foci. *B. melitensis* was isolated from the spleen and bonemarrow and the serum agglutinated *B. melitensis* 11, 1:1000, *B. abortus* 80, 1:800 and *B. melitensis* 7 and 9, <1:20.

Two guinea-pigs (706 and 707) inoculated with the culture obtained from the urine of guinea-pig 615 survived and presented negative necropsy findings. A third guinea-pig (708) died on the 30th day after the inoculation with typical lesions and positive cultural findings.

A second series of 12 guinea-pigs gave the following result: Four of 6 guinea-pigs inoculated intraperitoneally with 2,000 million bacilli of a mixture of *B. melitensis* strains 2, 3, 4, 5 and 11 died on the 2nd and 3rd days, respectively, after the inoculation. In every case a fibrinopurulent peritonitis was present, and the injected bacilli were isolated from every organ. The remaining 2 guinea-pigs proved negative at necropsy on the 120th day. Six guinea-pigs injected intratesticularly with the same inoculum survived and presented negative findings when sacrificed 112 days after the inoculation.

A third series of 4 guinea-pigs inoculated intraperitoneally with 1/50 slant of the mixture mentioned of strains furnished 2 guinea-pigs (807 and 809) with moderately severe chronic lesions. Two guinea-pigs died on the 16th and 26th day, respectively; *B. melitensis* was found in the spleen, liver, bonemarrow, bile, kidneys and urine, but not in the heart blood. The histories of guinea-pigs 807 and 809 are detailed.

Guinea-Pig 807.—Weighing 330 gm., was inoculated intraperitoneally with 1/50 slant of a mixture of *B. melitensis* 2, 3, 4, 5 and 11. It was chloroformed 87 days after the inoculation when it weighed 417 gm. The spleen weighed 3.5 gm., it was nodular and soft; the edges were rounded. Numerous lymphnodes, in particular the iliac, precrucial and postmanubrial nodes, were slightly enlarged and firm. A few *B. melitensis* were isolated from the splenic pulp; the bonemarrow, liver, bile, urine, and kidneys were sterile. The serum agglutinated the various strains as follows: *B. melitensis* 11, 1:100-200; *B. melitensis* 7, 1:80; No. 9, 1:20; and *B. abortus* 80, 1:200; after absorption with *B. abortus* 80, the serum agglutinated *B. melitensis* 1:60. The strain of

B. melitensis isolated from the spleen was agglutinated in a dilution of 1:200 by a group II serum after the serum had been absorbed with *B. abortus* No. 80 (serum titer 1:1000, after absorption 1:200 for *B. melitensis* group II and *B. abortus* 80, <1:40). An emulsion of the spleen was inoculated intraperitoneally or intratesticularly into guinea-pigs 877, 878 and 879.

Guinea-Pig 809.—Weighing 685 gm., was inoculated intraperitoneally as guinea-pig 807. It gave a strongly positive skin reaction with *B. melitensis* and *B. abortus* protein on the 76th day after the injection. The guinea-pig was sacrificed on the 97th day when it weighed 676 gm. The postmortem findings were typical; the spleen weighed 2.2 gm.; it was nodular and pulpy. The lymphnodes were enlarged and hard. *B. melitensis* was isolated from the spleen, while the urine, bile, bonemarrow, etc., were sterile. The serum agglutinated the various *B. melitensis* strains as follows: *B. melitensis* 11, 1:200; *B. melitensis* 7 and 9, 1:100; and *B. abortus* 80, 1:200; when absorbed with *B. abortus* No. 80, the serum agglutinated only *B. melitensis* 11 in a dilution of 1:100.

In a fourth series the infected spleen of guinea-pig 807 was injected intraperitoneally into guinea-pigs 877 and 878, while guinea-pig 879 was treated intratesticularly. The three animals presented typical lesions when sacrificed on the 92nd day. The abstracted histories are briefly as follows:

Guinea-Pig 877.—Weighing 575 gm., was injected intraperitoneally with 2 c.c. of an emulsion 1:10 of the spleen of guinea-pig 807. When sacrificed on the 92nd day, the guinea-pig weighed 526 gm. and showed a nodular, enlarged spleen (weight 1.5 gm.), numerous foci in the liver, enlarged and firm lymphnodes. *B. melitensis* was isolated from the spleen, kidneys and liver. The serum agglutinated *B. melitensis* 1, 1:400 and *B. abortus* 1:400; after absorption, *B. melitensis* 27 was clumped in a dilution of 1:100, *B. abortus* <1:80.

Guinea-Pig 878.—Weighing 526 gm., was inoculated as 877. The weight at necropsy was 566 gm.; the spleen was enlarged and nodular (weight 2.1 gm.); the uterus contained 3 embryos. *B. melitensis* was isolated from the left kidney, spleen and one placenta. The serum agglutinated strains 26 and 615 in dilution >1:200 after absorption with *B. abortus* 80. *B. melitensis* was agglutinated 1:160.

Guinea-Pig 879.—Weighing 593 gm., was inoculated intratesticularly as was guinea-pig 877. At necropsy (weight 563 gm.) the nodular spleen weighed 1.2 gm., and the left seminal vesicle was distended by stringy pus. *B. melitensis* was isolated from the spleen (6 colonies), urine and left kidney. The serum reactions were as follows: *B. melitensis* 21, 1:800; No. 11, 1:400; *B. abortus*, 1:100; No. 7 and 9, <1:10; after absorption with *B. abortus*, *B. melitensis* 11 was clumped 1:640 + + +.

EXPERIMENTS WITH *B. MELITENSIS* STRAIN 6

The strain used in these experiments was obtained in 1919 from Dr. E. Sergent, Institut Pasteur d'Algerie, and was marked "Race Souk-Alvias." Six guinea-pigs were inoculated with 1/10 slant of *B. melitensis* 6. One guinea-pig (639) died on the 10th day and one (638) was sacrificed on the 15th day. Both animals showed typical lesions, while the remaining 4 guinea-pigs, when sacrificed on the

140-160th day after the inoculation, exhibited normal necropsy findings. The necropsy findings of guinea-pigs 638 and 639 are detailed.

Guinea-Pig 638.—Weighing 675 gm., was inoculated into the right testicle with 1/20 slant of a young culture of *B. melitensis* 6. It was chloroformed 15 days after the injection, when it weighed 714 gm. Necropsy revealed a generalized enlargement of the lymphnodes and atrophy of the testis; the spleen weighed 0.5 gm., was light brownish and soft. *B. melitensis* was isolated from only the spleen and the right testis. The serum agglutinated *B. melitensis* 5, 1:100 + + +; while it failed to react with *B. abortus* 320.

Guinea-Pig 639.—Weighing 505 gm., was inoculated intratesticularly as was guinea-pig 638. The animal died on the 10th day (weight 460 gm.). The spleen weighed 1.4 gm., was nodular and showed numerous yellowish necrotic foci; similar focal necroses were seen in the liver and the iliac lymphnodes; the right testis was partially necrotized and deeply injected. Cultures demonstrated the fact that all the organs, bile and urine were teeming with *B. melitensis*.

EXPERIMENTS WITH *B. MELITENSIS* STRAIN 11

The strain was obtained in 1918 as *Micrococcus melitensis* 33 from the American Museum of Natural History.

Two series of (717-726 and 753-758) 5 and 6 guinea-pigs each were inoculated intraperitoneally and intratesticularly with 1/3-1/10 slant of a young agar culture. In the first series, only 2 animals, guinea-pig 720 (intratesticularly) and 724 (intraperitoneally), gave positive cultures at necropsy. In the second series, 5 of the 6 animals injected intraperitoneally died in from 4-12 days after the inoculation; they exhibited the usual lesions, the surviving animal was negative. Two guinea-pigs (753 and 755), infected by the intratesticular route, presented typical necropsy findings; one animal (754) had succumbed to an intercurrent infection; one (756) died 7 days after the injection, and 2 (757 and 758) gave negative findings. The histories of the infected guinea-pigs are detailed.

Guinea-Pig 720.—Weighing 615 gm., received intratesticularly 1/3 slant of *B. melitensis* No. 11. Skin tests were repeatedly positive. At necropsy examination (weight 870 gm.) 127 days after the inoculation, the spleen was small, weighing 0.9 gm., the right testis was hard and atrophic, numerous lymphnodes were slightly enlarged and firm; *B. melitensis* was isolated from the pus and spleen. The serum agglutinated *B. melitensis* 1:200 after absorption with *B. abortus*.

Guinea-Pig 724.—Weighing 462 gm., was inoculated as was 720. Two cutaneous tests were positive. Sacrificed on the 127th day (weight 722). The omentum was adherent to the liver and spleen, the spleen was small and weighed 0.9 gm. The precrural, postmanubrial and iliac lymphnodes were firm and enlarged; the testes were atrophic; *B. melitensis* was isolated from the precrural lymphnodes. The serum agglutinated *B. melitensis* 11 after absorption with *B. abortus* 1:80.

Guinea-Pig 753.—Weighing 607 gm., was injected into the right testicle with 1/10 slant of a young culture of *B. melitensis* 11. When chloroformed on the 112th day after the inoculation, it weighed 608 gm. and showed the following lesions: Spleen slightly enlarged, weight 1.4 gm., nodular; the precucular and iliac lymphnodes were hard and slightly enlarged; both testes were atrophic. *B. melitensis* was isolated from the right testis. The serum agglutinated *B. melitensis* 1:400 and *B. abortus* 1:100.

Guinea-Pig 755.—Weighing 613 gm., was inoculated in the same manner as guinea-pig 753. When sacrificed on the 112th day, it weighed 495 gm. and exhibited typical lesions. The spleen weighed 2.1 gm. and was distinctly nodular. The serum agglutinated several strains of *B. melitensis* as follows: *B. melitensis* 11, 1:600; *B. melitensis* 7 and 9, 1:20-1:40; *B. abortus* 80, 1:200. *B. melitensis* group II was isolated from the spleen.

EXPERIMENTS WITH *B. MELITENSIS* STRAIN NO. 18

The strain used in these experiments was obtained from the Lister Institute; it was marked *M. melitensis* (Arkwright) and had been isolated in Malta in 1915 or 1916 from the blood of a patient. Two series of 7 guinea-pigs were inoculated intratesticularly. Four animals died in from 2-15 days after the inoculation and revealed at necropsy the usual lesions of a septicemia. Three animals survived and 2 (766 and 812) presented typical anatomic and cultural findings.

Guinea-Pig 766.—Weighing 460 gm., received intratesticularly 1/10 slant of *B. melitensis* 18. Skin tests were positive on the 82nd day. Necropsy examination on the 119th day revealed an emaciated body (weight 382 gm.), an enlarged, nodular spleen, weighing 1.6 gm., firm and enlarged lymph nodes, atrophic right testis and purulent seminal vesiculitis. *B. melitensis* was isolated from the pus and the spleen. The serum was lost.

Guinea-Pig 812.—Weighing 510 gm., was injected as guinea-pig 766. At necropsy (98th day, weight 490 gm.) the spleen weighed 1.8 gm., and was distinctly nodular. *B. melitensis* was isolated only from the spleen. The serum agglutinated *B. melitensis* 18, 1:200; *B. abortus* 80, 1:1000; *B. melitensis* 7 and 9, 1:60, after absorption with *B. abortus* 80. *B. melitensis* was agglutinated 1:100 and *B. abortus* 80, <1:40.

EXPERIMENTS WITH *B. MELITENSIS* 19

The strain was received from the Lister Institute; the tube was marked *M. melitensis* Eyre—milk, malta fever.

Two series of 4 guinea-pigs each were inoculated intratesticularly. Two animals died in from 5-15 days after the inoculation. Six guinea-pigs survived and 5 presented at necropsy normal organs with negative cultural findings, while one (770) showed these lesions:

Guinea-Pig 770.—Weighing 483 gm., was inoculated with 1/10 slant of *B. melitensis* 19. Necropsy on the 110th day revealed numerous small abscesses in the right testis and epididymis and pus in the right seminal vesicle; the spleen was small (0.65 gm.); the lymphnodes were firm and slightly enlarged. *B. melitensis* was isolated only from the pus of the abscesses. The serum agglutinated *B. melitensis* 19 in a dilution 1:200.

EXPERIMENTS WITH *B. MELITENSIS* 20

The strain used in these experiments was obtained from the Lister Institute; the tube was marked *M. melitensis* Austria I, and it was stated that the culture was originally obtained from the Royal Army Medical College, Millbank, London. This strain belongs to our group 1 of the *Brucella* group and cannot be separated from the *B. abortus* group. In paper III the *melitensis* character of the organism was proved by a pathogenicity test on one monkey.

Two series of 4 guinea-pigs each were inoculated intratesticularly. One animal died on the 22nd day after the inoculation, the spleen appeared normal, but from it *B. melitensis* was isolated. Seven guinea-pigs were sacrificed in from 72-132 days after the infection; necropsy revealed no pathological changes.

EXPERIMENTS WITH *B. MELITENSIS* 21

The strain was obtained from the Lister Institute and was marked *M. melitensis* (Basset-Smith). According to a personal communication from Surgeon Rear-Admiral P. M. Basset-Smith, Royal Naval College, Greenwich S. E., this strain was isolated by him in 1904 from a blood culture of an undulant fever patient in his ward and sent to the Lister Institute in 1905. *B. melitensis* 21 belongs serologically to group 2 or the main group of the genus *Brucella*. The pathogenicity for guinea-pigs was tested on 3 series of animals. The first series of 4 guinea-pigs gave entirely negative results. The histories of the guinea-pigs used in the second series are presented:

Guinea-Pig 819.—Weighing 543 gm., was inoculated intratesticularly (right testis) with 1/10 slant of a young culture of *B. melitensis* 21. The animal was sacrificed on the 103rd day, when it weighed 445 gm. The anatomic lesions were typical; the spleen weighed 1.82 gm. (1.2 x 4.0 cm.), showed numerous grayish nodules and a deep brownish pulp; the lymphnodes were all enlarged and hard. In the kidneys a few grayish nodules were visible. The right and left testes were atrophic, the right was fibrous. *B. melitensis* was isolated from the spleen and kidneys. The serum of the guinea-pig agglutinated various strains of the *Brucella* group as follows: *B. melitensis* 21, 1:1000-2000; *B. melitensis* 7 and 9, 1:40-1:80; *B. abortus* 80, 1:800; absorbed with *B. abortus* 80, agglutinated *B. melitensis* 1:400 + + +.

Guinea-Pig 820.—Weighing 395 gm., was inoculated as guinea-pig 819; it was found dead 10 days after the inoculation (weight 254 gm.). The right testis was partially necrotic, covered with fibrin, the spleen was deep brownish, soft, slightly enlarged (0.9 gm.), and the bile was blood tinged. *B. melitensis* was isolated from the spleen, liver, urine and from the bile; innumerable colonies developed on the blood plates. No serum could be procured.

Guinea-Pig 821.—Weighing 383 gm. and treated as 819, died on the 8th day after the inoculation. The lesions and cultural findings were identical to those recorded for guinea-pig 820.

TABLE 1
EXPERIMENTS WITH B. MELITENSIS 21, SERIES 3

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings Serum Agglutinates
		Initial, Gm.	At Necropsy, Gm.				
883 ♂	1/25 slant	630	529	70th day	Spleen 2.1 gm., nodular and typical; lymphnodes enlarged and hard; foci in liver and lungs; testis atrophic, left adhesions microscopically typical Spleen 1.1 gm., both testis atrophic, left fibrous; lymphnodes slightly enlarged; 8-10th right and left sternocondral junction of ribs enlarged, nodular, containing a yellowish pus-like substance Lesions as above mainly in right ribs; spleen 0.9 gm. General lymphadenopathy; abscess in right testis and purulent seminial vesiculitis, atrophy left testis; spleen 0.9 gm. Cloudy swelling in parenchymatous organs	B. melitensis isolated from left femoral bonemarrow, spleen liver and precural lymphnode	B. melitensis 21, 1:2000; B. abortus 1:80 after absorptions with No. 80 agglutinates B. melitensis 21, 1:640, B. abortus 80, <1:20
884 ♂	1/25 slant	598	506	70th day		B. melitensis isolated from two rib lesions, spleen; liver and bonemarrow sterile	B. melitensis 1:1000; B. abortus 1:200, absorbed with B. abortus agglutinates B. melitensis 1:640
885 ♂	1/25 slant	637	515	70th day		B. melitensis isolated from ribs only	B. melitensis 1:1000; B. abortus 80, 1:160
886 ♂	1/25 slant	738	390 D.	41st day		B. melitensis isolated from testis and spleen	Not tested
887 ♂	1/25 slant	783	625	70th day	Spleen 1.4 gm.; 12th right rib enlarged peristostochondritis on left lower femur and upper end of tibia involving left knee. Lymphnodes not enlarged	B. melitensis isolated from spleen and rib lesion	B. melitensis 21, 1:400; B. abortus 1:400, after absorption, B. melitensis 21 1:200; B. abortus 80, <1:20
888 ♂	1/25 slant	585	595	70th day	Spleen 0.75 gm.; both testis atrophic and seminal vesicle fibrous; right and left sternocondral rib insertion enlarged as 884	B. melitensis isolated from spleen and rib lesions	B. melitensis 21, 1:2000; B. abortus 80 after absorption; B. melitensis, >1:1280; B. abortus <1:20

The exceedingly interesting and important results collected from series 3 are presented in table 1.

EXPERIMENT WITH *B. MELITENSIS* 22

The strain is known as *B. paramelitensis* (Basset-Smith) originally sent to Fleet Surgeon R. W. Basset-Smith by Dr. C. Nicolle of the Institut Pasteur de Tunis, obtained from the Lister Institute. Seven guinea-pigs were tested with this strain. Four animals died in from 10-21 days showing the usual lesions of a septicemia. Only one (822) of the 3 surviving guinea-pigs presented typical lesions at necropsy, 79-120 days after the inoculation.

Guinea-Pig 822.—Weighing 685 gm., was inoculated intratesticularly with 1/10 slant of *B. melitensis* 22. The same animal gave a strongly positive skin reaction and was therefore sacrificed on the 79th day after the injection (weight 538 gm.). The spleen was enlarged and weighed 1.4 gm. (2.5 x 1.5 cm.); the lymphnodes were small but hard; the left seminal vesicle contained creamy pus, and the liver exhibited a few foci. *B. melitensis* was isolated from the urine, spleen, seminal vesicle and both kidneys. The serum gave the following serum reactions: *B. melitensis* 22, 1:800 + + +; *B. melitensis* 11, 1:100 and *B. abortus* 80, 1:800 + + +.

EXPERIMENTS WITH *B. MELITENSIS* 26

The strain was obtained from Dr. J. W. H. Eyre, Guy's Hospital, London; it had been isolated in 1918 (?) from the spleen of a case of undulant fever. The results obtained on 9 guinea-pigs are detailed:

SERIES 1.—*Guinea-Pig 834*.—Weighing 480 gm., was inoculated intratesticularly with 1/10 slant of *B. melitensis* 26. The animal was sacrificed on the 108th day after the inoculation (weight 355 gm.) and revealed typical lesions. The spleen weighed 1.4 gm. (0.8 x 2.2 cm.), and the lymphnodes were slightly enlarged. The right testis was atrophic and fibrous. *B. melitensis* was isolated from the spleen. The serum agglutinated the various available strains as follows: *B. melitensis* 26, 1:1000 + + +; *B. abortus* 80 and *B. melitensis* 7 and 9 in a dilution of 1:40.

Guinea-Pig 835.—Weighing 440 gm., was inoculated in the same manner as guinea-pig 834. The animal died on the 25th day after the inoculation (weight 320 gm.); the right testis contained an abscess; the regional lymphnodes were enlarged and firm; the spleen was small (0.5 gm.). *B. melitensis* was isolated from the pus of the testicular abscess, the bonemarrow, the bile and the spleen. No serum was obtained for agglutination tests.

Guinea-Pig 836.—Weighing 360 gm., was inoculated intratesticularly with 1/10 slant of *B. melitensis* 26. The animal died on the 28th day; it was emaciated and weighed 225 gm. Necropsy revealed a spleen weighing 1.3 gm., which was soft and light brown. The lymphnodes appeared normal, while the testes were atrophic; the right testis in particular was rather fibrous. The right epididymis contained creamy pus. The gallbladder was distended and contained blood tinged bile. *B. melitensis* was isolated from the spleen, epididymis, bile and left femoral bonemarrow. No serum could be procured for agglutination tests.

TABLE 2
EXPERIMENTS WITH *B. MELITENSIS* 26, SERIES 2

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings Serum Agglutinates
		Initial, Gm.	At Necropsy, Gm.				
889 ♂	1/20 slant	526	405	70th day	Spleen 2.5 gm.; (3.3 × 2.2) typical lymphadenitis; atrophic testis; foci in liver	<i>B. melitensis</i> isolated from spleen and bonemarrow	<i>B. melitensis</i> 26, 1:400; <i>B. abortus</i> 80, 1:100, absorbed with <i>B. abortus</i> 80, <i>B. melitensis</i> 1:300; <i>B. abortus</i> <1:40 Not tested
890 ♂	1/20 slant	873	833	Died 13th day	Spleen pulpy, 1.4 gm.; necrosis of right testis; cloudy swelling of liver and kidneys	<i>B. melitensis</i> isolated from spleen, liver, kidneys, bile, bonemarrow	
891 ♂	1/20 slant	463	541	78th day	Spleen 1.6 gm.; one nodule; slight lymphadenopathy; right testis atrophic	<i>B. melitensis</i> only in spleen.....	<i>B. melitensis</i> 1:200; <i>B. abortus</i> 80, 1:100; absorbed <1:40
892 ♂	1/20 slant	519	448	61st day	Spleen small, 0.8 gm.; slight enlargement of sternochondral junction of right 5th, 6th, 7th and 8th rib; other organs normal. Right testis small focus of necrosis	<i>B. melitensis</i> only from right testis	<i>B. melitensis</i> , 1:60; <i>B. abortus</i> 80, 0
893 ♂	1/20 slant	618	603	Died 6th day	Hemorrhagic orchitis; intoxication	<i>B. melitensis</i> isolated from spleen, liver and bile; no other organ tested	Not tested
894 ♂	1/20 slant	513	475	70th day	Spleen 2.4 gm., typical; lymphadenopathy; atrophic right testis; abscess in left epididymis; foci in liver	<i>B. melitensis</i> isolated from spleen and seminal vesicle	<i>B. melitensis</i> 26, 1:1000; <i>B. abortus</i> 80 absorbed; <i>B. melitensis</i> , 1:400C-600; <i>B. abortus</i> 80, 1:40

EXPERIMENTS WITH *B. MELITENSIS* 27

The strain used in these experiments was obtained from Dr. T. W. H. Eyre, Guy's Hospital, London; the culture had been isolated in 1917 or 1918 from goat's milk. The results obtained on 8 guinea-pigs are detailed:

SERIES 1.—Guinea-Pig 837.—Weighing 490 gm., was inoculated intratesticularly with 1/10 slant of *B. melitensis* 27. The animal died on the 57th day when it weighed 317 gm. The lesions were typical. *B. melitensis* was isolated from the spleen (∞ colonies), right testis and seminal vesicle and the bile.

Guinea-Pig 838.—Weighing 428 gms., was inoculated in a similar manner as guinea-pig 837. It was found dead on the 57th day, when it weighed 249 gm. The lesions resembled those recorded for guinea-pig 837. *B. melitensis* was isolated from the spleen, right testis and the bile.

Guinea-Pig 840.—Weighing 728 gm., was inoculated intratesticularly with 1/10 slant of *B. melitensis* 27. On the 76th day after the inoculation, the animal gave strongly positive skin reactions with *B. abortus* and *B. melitensis* protein. The guinea-pig was sacrificed on the 99th day, when it weighed 694 gm. A typical nodular, enlarged spleen, weighing 2.3 gm. (3.2×2.5 cm.) was found. The liver was studded with numerous grayish areas, and all the lymphnodes were slightly enlarged and firm. The right testis and seminal vesicle were atrophic. *B. melitensis* was isolated from the spleen (∞ colonies), urine and left femoral bonemarrow. The agglutination reactions with the serum were as follows: *B. melitensis* 27, 1:200; *B. abortus* 1:100; *B. melitensis* 7 and 9, 1:20-40; after absorption with *B. abortus* 80, the serum agglutinated *B. melitensis* 1:100.

Guinea-Pig 881.—Was inoculated intraperitoneally with 2 cc of a spleen emulsion of guinea-pig 840. When sacrificed on the 103rd day, the normal spleen weighed 0.91 gm.; the lymphnodes were slightly enlarged, and the uterus presented an endometritis. *B. melitensis* was isolated from the spleen (31 colonies). The serum agglutinated *B. melitensis* 11, 1:400, after absorption 1:160.

EXPERIMENTS WITH *B. MELITENSIS* 29

The strain was obtained in 1919 from Dr. Guido Isar, Catania, Sicily, and was originally isolated from a case of human undulant fever. Five guinea-pigs were inoculated intratesticularly. Four animals were found free from anatomic lesions and gave negative agglutination reactions when sacrificed 72 days after the inoculation. The history of one animal (1001) is presented:

Guinea-Pig 1001.—Weighing 515 gm., was injected into the right testicle with 1/5 slant of *B. melitensis* 29. The animal presented a rough coat and gave a positive skin reaction. It was sacrificed on the 72nd day after the inoculation, when it weighed 515 gm. Necropsy revealed a small spleen (0.62 gm.); enlarged and firm precrucal, iliac and peri-aortic lymphnodes (size of small peas); the left seminal vesicle contained watery pus; the right testis was atrophic and fibrous; the right radiocarpal region was enlarged and showed the typical fibrous thickening about the joint. *B. melitensis* was isolated from the urine, seminal vesicle, spleen and radiocarpal region. The serum agglutinated *B. melitensis* 29, 1:400; *B. abortus* 80.

TABLE 3
EXPERIMENTS WITH *B. MELITENSIS* 27, SERIES 2

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings Serum Agglutinates
		Initial, Gm.	At Necropsy, Gm.				
885	1/25 slant	393	270	Died 10th day	Lung edema, hemorrhagic orchitis...	B. melitensis from spleen, testis, but not heart blood	Not tested
886	1/25 slant	389	470	70th day	Spleen 1.8 gm.; distinctly nodular several lymphnodes enlarged. Testis atrophic	Few B. melitensis from spleen...	B. melitensis 1:400; B. abortus 80, 1:200 absorbed; B. melitensis 1:100, B. abortus <1:40
897	1/25 slant	424	507	70th day	Spleen 1.2 (3.2 x 1.8 cm.) two nodules; lymphnodes enlarged	Few B. melitensis colonies from spleen	B. melitensis 27, 1:600; B. abortus 80, 1:400; absorbed; B. melitensis 1:100; B. abortus <1:40
898	1/25 slant	443	280	Died 9th day	Right testis necrotic; numerous foci in liver and spleen. Intoxication	B. melitensis from spleen, liver and testis	Not tested

The essential facts dealing with the number of guinea-pigs used for each strain, the course of the infection, the anatomic lesions noted at necropsy and the cultural and serologic results are summarized in table 4. It is shown that 12 strains of *B. melitensis* inoculated by the intratesticular or intraperitoneal route in amounts varying from 2,000-3,000 millions failed to produce a definite disease, which could be identified either by anatomic lesions or by positive cultures. On the other hand, 4 cultures (11, 21, 26 and 27) which were of human or caprine origin proved highly pathogenic for guinea-pigs, while 6 other strains (6, 19, 18, 20, 22 and 29) and a mixture of 5 strains (2, 3, 4, 5 and 11) occasionally caused lesions.

TABLE 4
SUMMARY OF EXPERIMENTS WITH STRAINS OF *B. MELITENSIS*

Strains	Number of Guinea-Pig Inoculated	Number of Infections	Course of Infection		Anatomic Lesions						Cultures	Serum Reactions
			Acute and Fatal	Sub-acute and Chronic	Lymph-nodes	Spleen	Liver	Lung	Testis Abscesses	Bones		
1, 2, 3, 4, 5, 7, 8, 9, 10, 23, 24 and 25....	60	0	—	—	—	—	—	—	—	—	—	—
2, 3, 4, 5 and 11.....	41	21	7	14 (44-155)	12	14	9	1	3	—	21	14
6 (Algiers human ?)...	6	2	2	—	2	2	2	—	—	—	2	+2
11 (English human 1896)	31	11	6	5	4	2	1	0	1	0	11	+4
18 (England human 1915)	7	6	4	2	1	2	0	0	1	0	6	+2
19 (England goat).....	8	3	2	1	0	0	0	0	1	0	3	1
20 (Austria human ?)...	8	1	1	—	—	—	—	—	—	—	1	—
21 (England human 1904)	13	9	2	7	5	5	0	1	2	4	9	7
22 (Para melitensis).....	7	5	4	1	1	—	1	—	1	—	5	1
26 (England human 1918)	9	9	2	7	2	4	2	—	4	1	9	5
27 (England goat 1917)...	8	8	2	6	6	6	1	0	(2)	0	8	4
29 (Sicily human ?)...	5	1	0	1	1	0	0	0	1	1	1	—
	203	76	32	44	34	35	16	2	14 (2)	6	76	40

SYMPTOMS AND COURSE OF INFECTION

The testicle, which had been inoculated with the bacterial suspension, became quickly swollen and tender, and the scrotum developed a purple reddish color. In a few animals, local ulcerations were noted. As a rule, the guinea-pigs refused their food for several days, were visibly sick and lost from 100-200 gm. of weight. The temperature was

elevated in most instances and varied between 38-40.2 C. Muscular weakness, ruffled coat and tonic spasms usually preceded death from an acute infection. The train of symptoms and the postmortem findings in the 32 instances differed in no way from those described by Eyre,⁸ following intracranial injections. Severe intoxications were common in animals infected by the intraperitoneal route. Thirty-two of the 76 successfully infected guinea-pigs died in from 2-20 days. As the experiments reported in this paper were primarily undertaken for the study of subacute and chronic lesions similar to those seen in guinea-pigs infected with *B. abortus*, all the animals which died in the first 20 days after the injection presented lesions of relatively little interest and are in this presentation classified as having acute, fatal infections. Guinea-pigs suffering from subacute or chronic *B. melitensis* infections manifested in the first few days after the injection certain of the symptoms already described. The loss in weight was distinct, and later in the course of the disease a rough coated appearance was frequently present, particularly in animals with marked focal lesions. Swelling of the radiocarpal and knee joints was observed in a series of guinea-pigs inoculated with strain 21 and in one animal infected with strain 29.

As regards the course of the disease, the animals divided themselves into 4 groups. Some ran a gradually downward course, became much emaciated and died between the 25th and 50th day after the injection. Larger animals regained their loss slowly and, when sacrificed on the 79th to the 119th day after the injection of 1/10-1/25th of a slant, had nearly reached the original weight, while others registered a loss of from 18-179 gm. In a small number of guinea-pigs, the normal physiologic increase in weight continued, and the animals were disposed of at the end of from 70 to 155 days, in excellent condition. The course of the infection was acute and fatal in 42% and subacute or chronic in 58% of the successfully infected guinea-pigs. Only 76 of the 143 animals infected with 10 different strains presented evidence, either in the form of positive skin tests or in macroscopic and microscopic lesions and positive cultures, which could be considered indicative of an infection. It is evident from these results that certain guinea-pigs have a greater resistance to *B. melitensis* than others; in fact, some of the experiments even suggested that the animals procured from certain breeders were nonsusceptible to a *B. melitensis* infection. This is the only apparent explanation which can be offered for the many failures which were obtained in several experimental series. In connection with

the experiments on *B. abortus*, more attention will be paid to this phase of the problem.

Of the 44 animals with chronic infections, 7, or 15.9%, died. Death was due to anaphylaxis in one, enteritis in another, and in the remaining 5 it followed emaciation and chronic intoxication.

PATHOLOGIC ANATOMY

The 32 animals which died in from 2-20 days following the intratesticular or intraperitoneal injection of various strains of *B. melitensis*, revealed either a necrotizing orchitis or a localized or diffuse fibrinopurulent peritonitis. The liver, spleen and kidneys were dark and engorged with blood; as a rule, the lymphnodes were enlarged and soft. In a few instances, small scattered necroses were visible in the liver. Invariably the inoculated bacteria were isolated from every organ, occasionally also from the heart blood.

The gross pathologic changes in the 44 guinea-pigs, which lived from 47 to 155 days, were in brief:

The lymphnodes were enlarged in 34 cases, or 77.2%. In about one-half of this number, the enlargement was confined to certain groups of nodes; thus the precucral and iliac groups were often quite conspicuous in the anemic inguinal and retroperitoneal fat layers. The firm and grayish nodes varied in size from that of a small grain to that of a small split pea.

The spleen was enlarged in 35 cases, or 79.5%. It should, however, be emphasized that the organ rarely attained the dimensions which were customarily observed in guinea-pigs infected with *B. abortus*. The average weight of the spleen of 17 infected and carefully weighed guinea-pigs, which lived for 70-155 days, was 1.7 gm. (average body weight 532 gm.). The maximum weight of the spleen was 3.5 gm., for an animal of 417 gm. Not infrequently the dimensions of the organ appeared normal, and without cultural or serologic tests the existence of an infection would have been overlooked. The appearance of the surface under the capsule was always nodular and speckled with grayish foci varying considerably in size, although large whitish areas, as seen in *B. abortus* spleens, were not observed in this experimental series. When definitely enlarged, the spleen was always darker than normal and presented a moist protruding pulp, with distinct foci on section. In 9 guinea-pigs, even though the spleen presented an apparently normal appearance, definite lesions were found in the genital organs or the ribs.

The liver in 16 guinea-pigs, or 36.6%, showed scattered over the surface either a few or many grayish, pin point foci. These lesions differed in no respect from those noted in guinea-pigs infected with *B. abortus*. In 3 guinea-pigs, which died on the 25th, the 28th and the 57th day, respectively, the gall-bladder contained a small amount of blood tinged bile, which was teeming with *B. melitensis*.

Only one guinea-pig revealed grayish foci in both kidneys on stripping the capsule; the genital organs were free from suppurative process in this case. The testes of all animals injected by the intratesticular route showed changes in the form of atrophy and fibrous degeneration of the parenchyma. In 9 instances the organs contained small or large abscesses. The diseased testicle was invariably adherent to the scrotal sac. The epididymis of the testicle into which the bacterial growth had been injected presented abscesses in 2 cases, while the opposite organ was involved in one instance. The seminal vesicle of

the infected right testicle contained creamy or thin liquid pus in 6 cases. In 3 instances, the left seminal vesicle was diseased and attached to a slightly atrophic and fibrous epididymis and testicle. The injections were always made in the right testicles. A striking bilateral atrophy of the testes was recorded in 9 animals.

The placenta of a guinea-pig with a triple pregnancy showed flakes of purulent exudate, and *B. melitensis* was isolated in culture from this material. Another pig with splenic lesions exhibited a chronic endometritis, but gave only a positive culture from the spleen.

The lungs of 2 guinea-pigs, or 4.5%, showed subpleural, translucent foci in the principal lobes. Five guinea-pigs inoculated with strain 21 presented marked swellings of the osteochondral junctions of several ribs. One animal had an involvement of the knee region and another, infected with strain 29, exhibited an enlargement of the right radiocarpal region. The lesions were identical with those described by Fabyean¹⁴ for guinea-pigs suffering from abortion disease.

The histologic changes were, in accordance with the macroscopic changes, either extensive or slight and could sometimes be found only after careful search of numerous sections. Common to all focal lesions in the lymphnodes, spleen and liver were the nests of epithelioid and scattered giant cells, frequently accompanied by a marked connective tissue hyperplasia. The pathologic process is indistinguishable from that seen in guinea-pigs infected with *B. abortus*.

CULTURAL RESULTS

Systematic preparations of cultures disclosed an interesting distribution of *B. melitensis* in the infected guinea-pigs. Positive cultures were obtained from:

Spleen	33 animals
Testicles, abscesses in epididymis and pus in seminal vesicles	14 animals
Bonemarrow of left or right femur.....	6 animals
Kidneys and bile.....	5 animals
Precurral lymphnodes and liver.....	4 animals
Bone lesions	4 animals
Urine	3 animals
Placenta	1 animal

It will be noted that the spleen furnished positive cultures more frequently than any other organ. In this connection, it should be stated that the number of colonies which grew on the blood plates were in many cases less than 10. Such cultures were often obtained from animals which were sacrificed after a period of observation of more than 100 days. When *B. melitensis* could not be isolated from the spleen, either the suppurative processes in the genital organs or the bonemarrow and lymphnodes furnished positive cultures. The urine gave cultures of *B. melitensis* only in 3 instances. These findings are in contrast to those of Durham, of Eyre and of Nicolle and Conseil, who obtained a fairly good growth from the secretion in about 50% of the animals dying from a chronic infection. The cultures procured from the tissues were readily classified by agglutination tests, and, as far as these methods permit correct deductions, it can be concluded that only descendants of the injected strains were recovered. One culture obtained from guinea-pig 655 was tested on a monkey and proved to be a typical strain of *B. melitensis*.

¹⁴ Jour. Med. Res., 1912, 26, p. 441.

SEROLOGIC RESULTS

The experiments dealing with this phase of the problem have been reported in paper II of this series. The serum of the infected guinea-pigs agglutinated in a dilution of 1:200-1:2,000 those cultures belonging to the same group of *B. melitensis* as that used for the infection. As a rule, the reactions were distinct and of diagnostic value. Animals exhibiting no gross lesions and no positive cultures failed to agglutinate either *B. melitensis* or *B. abortus* in dilutions above 1:40. Serum tests may give results indicative of an infection, if performed shortly after specific antigens have been used for skin tests. Most of the serums agglutinated *B. abortus*; in fact, in some instances the latter organism was clumped in higher dilutions than the infecting *B. melitensis* strain. Absorption tests, however, invariably removed the coagglutinins and thus established the true nature of the infection.

GENERAL AND LOCAL HYPERSENSITIVENESS

The intracutaneous injection of bacterial proteins prepared from *B. abortus* and *B. melitensis* cultures produced in the infected guinea-pigs marked skin reactions; in one animal (655) death followed the introduction of the protein preparations in less than 24 hours. Anaphylactic symptoms were noted and lesions of an acute intoxication were present in the form of congestion and small hemorrhages in the viscera.

It was impossible to diagnose the type of infection with the aid of the skin tests. "Aborto" as well as "melitensis protein" produced indurated areolae varying in size from 1.4-1.8 cm., although the existence of disease was definitely indicated in the animals which were not emaciated. Guinea-pigs with local suppurative processes in the seminal vesicles or testicles responded by small indurations; while generalized infections were conducive to large and pronounced skin reactions. Cutaneous hypersensitiveness tests have proved invaluable in segregating the experimental animals. The method deserves more frequent application than it has received hitherto.

SUMMARY

The data presented in the preceding paragraphs indicate that some strains of *B. melitensis* are capable of causing lesions in guinea-pigs, which are sometimes remarkably similar to those produced by *B. abortus* in this species of animals. Four cultures, 2 old strains isolated from human cases of undulant fever in 1896 and 1904; 1 human strain isolated in 1915 and 1 caprine strain recovered from goat's milk in 1917 infected guinea-pigs fairly regularly, while 18 other strains either proved nonpathogenic or occasionally produced lesions in the spleen, lymphnodes, etc. It is noteworthy that strain 20, which, morphologically, biochemically and serologically cannot be separated from the *B. abortus* group, caused acute infections, but thus far no chronic lesions have been observed. The pathogenicity of *B. melitensis* strains for guinea-pigs is influenced neither by the origin nor the age of the culture. The experiences collected in this paper suggest that the capacity of this organism to infect guinea-pigs is a property possessed by only a few strains, although the observations of previous workers strongly indicate

that recently isolated cultures or body fluids containing *B. melitensis* will usually produce distinct lesions. Individual susceptibility of the guinea-pigs may be an important factor. On 2 occasions, only 1 guinea-pig of 8 injected with cultures which had been recently isolated from definite lesions developed a chronic infection, while animals procured from another breeder and inoculated with the same strain became diseased and exhibited interesting rib lesions in 5 out of 6 cases. The tissues most frequently attacked are the spleen and lymphnodes. Lesions in the liver, lungs, kidneys and bones have also been observed. It should be emphasized, however, that the pathologic processes in the spleen and lymphnodes of *B. melitensis* guinea-pigs appear less striking than those seen in this laboratory in animals infected with *B. abortus*. In fact, the impression has been gained that *B. melitensis* strains are less invasive for guinea-pigs than *B. abortus*. It is also recalled that the first series of intraperitoneal infections showed at necropsy perfectly normal viscera, which negative results prompted subsequent use of the intratesticular route of inoculation. Sufficient data are now available to justify the conclusions that in all probability injection by the intraperitoneal or subcutaneous route, or even feeding infections, may cause specific and characteristic lesions, provided certain strains and susceptible guinea-pigs are chosen. Recently isolated strains and particularly cultures or milk specimens procured from infected goats should be tested on guinea-pigs to determine their pathogenic properties.

EXPERIMENTS WITH *B. ABORTUS*

Since Theobald Smith and Marshall Fabyean¹⁵ called attention to the lesions produced in guinea-pigs after injections with *B. abortus*, several workers have studied this interesting inoculation disease. The publications of Fabyean,¹⁴ Schroeder and Cotton,¹⁶ Fleischner and Meyer¹⁷ and Smillie¹⁸ recorded definite gross changes in the viscera, enlarged, nodular spleens, lymphadenopathy, atrophy of the testicles, induration and suppuration of the epididymis, minute foci in the liver, etc. In the majority of instances, these lesions followed the injection of milk, infected cotyledons or cultures recently isolated from diseased guinea-pigs. After careful perusal of the American reports, one cannot escape the impression that splenic lesions are always present and that positive infections occur with remarkable regularity, provided the material inoculated contains a sufficiently large number of abortion bacilli. Many years ago one of us (K. F. M.), experimenting with old stock cultures of *B. abortus*, repeatedly failed to infect guinea-pigs, and for this reason the literature was carefully scrutinized for information which might explain the failures. As some of these references are nearly inaccessible, an attempt is here made to review this data.

¹⁵ Centralbl. f. Bakteriöl., 1912, I, 61, p. 549.

¹⁶ Twenty-Eighth Annual Report Bureau of Animal Industry, 1911, p. 139.

¹⁷ Amer. Jour. Dis. Child., 1917, 14, p. 157.

¹⁸ Jour. Exper. Med., 1918, 28, p. 585.

T. C. Evans,¹⁹ of Canada, described in 1914 the lesions occurring after the inoculation of guinea-pigs with infected milk. His description agrees with that of Schroeder and Cotton, with the reservation that the lesions he found were less pronounced. He ascribed the differences to lack of resistance of his guinea-pigs on account of the cold quarters in which they were kept, or the more marked virulence of the Canadian strains. Most of his guinea-pigs died from the infection, which must, therefore, have been virulent, as most workers have found that death is not a usual sequel.

Krage²⁰ reported, in 1913, that he had inoculated 36 guinea-pigs subcutaneously, intraperitoneally and intramuscularly with 0.5-2.0 c.c. of a culture of *B. abortus*, which was virulent for mice. One animal died from peritonitis; 8 succumbed to intercurrent infections. The remainder were sacrificed at the end of 8 months following the infection. No macroscopic lesions were found, but the serum of the animals agglutinated *B. abortus* in a dilution of 1:1,000. In another series of 16 guinea-pigs, he injected 4 strains, highly virulent for mice, subcutaneously and intraperitoneally. Three animals died on the second day and 3 during the second and third month; one presented a hemorrhagic metritis. The remaining 10 were sacrificed at the end of 6 months. *B. abortus* was isolated from the precaval lymphnode of an intraperitoneally injected animal; the serum agglutinated *B. abortus* in a dilution 1:4,000. Two other guinea-pigs gave positive cultures from subcutaneous abscesses; one of these animals presented a slightly enlarged spleen. The serum of both guinea-pigs agglutinated *B. abortus* in a dilution of 1:100-1:400. The serum agglutination titer of the 7 guinea-pigs, which furnished no cultures, varied from 1:40-1:200.

In a third series, he injected subcutaneously and intraperitoneally the milk of goats, which had been artificially infected and which contained culturally *B. abortus*. Two died on the fourth day from peritonitis; 10 succumbed to intercurrent infections and only 4 of the remaining 26, at the end of from 5-6 months, gave serum reactions from 1:100-1:400. Tissue cultures were entirely negative.

Thomsen²¹ reviewed, in 1915, the observations which had been made by Holth, in C. O. Jensen's laboratory in Copenhagen. In 1912, Holth injected subcutaneously guinea-pigs, with 1 and 2.5 c.c. of an agar slant of recently isolated strains of *B. abortus*. Two animals, sacrificed on the 44th day after inoculation, presented subcutaneous abscesses, which contained bacterioscopically and culturally *B. abortus* in pure form. One animal presented an enlarged nodular spleen and typical lesions in the ribs, while the viscera of the other guinea-pig appeared normal. The third animal, which died from pneumonia on the 264th day, and the fourth, sacrificed on the 296th day, revealed normal organs at necropsy. In a second series, he injected into 2 guinea-pigs $\frac{1}{2}$ of an agar slant of an old laboratory strain subcutaneously and intraperitoneally; a third animal received $\frac{1}{4}$ of a slant intraperitoneally. The first guinea-pig died with a loss in weight of 90 gm. at the end of 14 days and exhibited numerous abscesses in the peritoneal cavity, testicles, etc.; the second guinea-pig died on the 11th day with a loss of 190 gm. in weight; it had diarrhea, an enlarged spleen and testicles; a pronounced infiltration was noted at the site of the inoculation. The third guinea-pig died on the 36th day in an extremely emaciated state (loss in weight 320

¹⁹ Report of the Director General for the Canadian Veterinary Service, 1914. No. 5 and No. 6, Reports of the Director of Veterinary Research, Union of South Africa, Pretoria, 1919, p. 357.

²⁰ Zentralbl. f. Bakteriologie, Ref., 1913, 57, p. 304.

²¹ Maanedsskrift for Dyrlaeger, 1915, 27, pp. 13 and 16.

gm.); necropsy revealed abscesses in the omentum, on the surface of the liver and in the testicles; numerous ribs and both carpal joints showed typical swellings.

Thomsen, in 1912, inoculated 13 guinea-pigs with fresh milk procured from cows which had aborted and which also gave positive serum reactions for *B. abortus*. Two guinea-pigs of this series gave cultures for *B. abortus* from a subcutaneous abscess in one and a splenic nodule in another.

In 1919, Robinson,²² in South Africa, detailed his studies on about 200 guinea-pigs, which were inoculated with infected milk or other material and killed at different periods after inoculation, varying from 4 weeks to 7 months. He found that only an enlargement of the spleen occurred with any degree of constancy—liver lesions were seen in only 3 cases. No changes were noted in the bones. Furthermore, Robinson concluded that the agglutination test applied to the serums of infected guinea-pigs is of very great use, particularly when the spleen is only slightly, if at all, enlarged and not abnormal in appearance. In his experience it seemed advisable to determine the question of infection in guinea-pigs by a combined study of the macroscopic lesions, the histologic examination and the agglutination test.

Zeller²³ injected guinea-pigs and rabbits intravenously, intraperitoneally and subcutaneously with large doses of *B. abortus* and *B. melitensis*. The necropsy findings of the animals sacrificed after varying time intervals were mostly negative. In a few cases, an enlargement of the spleen and mesenteric lymph-nodes was noted, and the injected bacteria were isolated from the spleen, uterus, testicles, urine, milk and, occasionally, the kidneys.

The findings of Krage, Holth and Zeller deserve consideration in a comparative study of the pathogenicity of *B. abortus* and *B. melitensis*. In fact, their results may explain some of the failures to produce gross lesions in guinea-pigs injected with *B. melitensis* cultures. Sufficient data had been collected in this laboratory to confirm the work of T. Smith and Fabyean, Schroeder and Cotton and others on *B. abortus* infections following the injection of infected milk or tissues of diseased guinea-pigs. Little was known, however, with regard to the effect of injecting old and recent stock cultures. According to the reports of T. Smith and Fabyean, cultures appear equally as virulent for guinea-pigs as infected material derived from cattle. Their tests were limited, however, to 2 old stock cultures, using 11 guinea-pigs. For comparison, these observations were not considered sufficiently comprehensive and a series of experiments was therefore undertaken (1) to compare the pathogenicity of old *B. abortus* stock cultures with that of *B. melitensis* cultures when applied by the subcutaneous or by the intratesticular route; (2) to study the lesions produced by recently isolated strains, and (3) to correlate the data collected from studies I and II with the findings on guinea-pigs which had been

²² No. 5 and No. 6, Reports of the Director of Veterinary Research, Union of South Africa, Pretoria, 1919, p. 357.

²³ Berl. tierärztl. Wechnschr., 1920, 36, p. 345.

TABLE 5
EXPERIMENTS WITH B. ABORTUS 80, SERIES 3

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings
		Initial, Gm.	At Necropsy, Gm.				
630	1/10 slant	722	642	15th day (moribund)	Lymphadenitis; splenic tumor 2.2 gm.; foci in liver, orchitis	Spleen, liver, testis gave positive cultures	B. abortus, 1:400; B. melitensis 11, 1:80
631	1/10 slant	730	533	23d day (moribund)	Spleen 3.1 gm.; lymphadenitis; foci in liver and lungs; cholecystitis, lesions in numerous ribs; conjunctivitis and opaque cornea	Spleen, bile, liver, urine, lymph nodes, bonemarrow	B. abortus, 1:2000; B. melitensis 5, 1:1000
632	1/10 slant	740	547	44th day, died in hypersensitivity test	Typical lesions; spleen 4.5 gm.	Spleen, liver, but not blood.....	Not tested
633	1/10 slant	709	465	90th day	Spleen 0.6 gm.; one rib junction right side enlarged; slight lymphadenitis; testes atrophic	Only rib lesion.....	B. abortus 80, 1:200; B. melitensis 11, 1:200
634	1/10 slant	825	797	183d day, negative skin reaction	Spleen small, 0.5 gm.; right testis atrophic	Sterile organs	B. abortus, 1:40; B. melitensis, <1:10
635	1/10 slant	680	292	Died 24th day	Toxemia; spleen, 1.4 gm.; lymph nodes hemorrhagic	Every organ, except heart blood	Not tested

injected with milk or other infected material. These experiments are briefly described in subsequent paragraphs.

INTRATESTICULAR AND SUBCUTANEOUS INOCULATIONS OF STOCK CULTURES OF *B. ABORTUS*

EXPERIMENTS WITH *B. ABORTUS* 80

(A) Six guinea-pigs were inoculated intratesticularly with 0.1 c c of an agar slant of a young culture of *B. abortus* 80; the strain was originally isolated from certified milk and was about 2 years old. The results obtained in this experiment are shown in table 5.

(B) Four guinea-pigs were inoculated subcutaneously with the same amount and suspension of *B. abortus* 80, as series A. The results were briefly as follows:

Guinea-Pig 802.—Weighing originally 580 gm., gave positive skin tests and, when sacrificed on the 87th day, weighed 570 gm.; the spleen (3.5 gm.) was nodular; the lymphnodes were small, but firm; few foci were noted in the liver. The cultures made from the spleen and liver were negative, but the serum agglutinated *B. abortus* in a dilution of 1:1,000.

Guinea-Pig 803.—Weighing 612 gm.; the skin tests were positive. When sacrificed on the 87th day, the animal weighed 512 gm. and had a small spleen, 0.9 gm.; the lymphnodes were small and soft. *B. abortus* was isolated from a small abscess in the right uterine horn and the lateral ligament. The blood serum agglutinated *B. abortus* in a dilution of 1:600.

Guinea-Pig 804.—Weighing 511 gm., and gave positive skin reactions. It was sacrificed on the 87th day, when it weighed 485 gm. The spleen was small and weighed 1.2 gm. All the organs appeared normal, and the cultures remained sterile. The serum agglutinated *B. abortus* in a dilution of 1:1,000.

Guinea-Pig 805.—Weighing 570 gm. and, when sacrificed, 521 gm. (87th day). The spleen was small, 0.6 gm. From the uterus, showing an endometritis with a retained, necrotized placenta, *B. abortus* was isolated. The serum agglutinated *B. abortus* in a dilution of 1:2,000.

EXPERIMENTS WITH *B. ABORTUS* 38

The strain was obtained from the Laboratory of the Pennsylvania State Live Stock Sanitary Board; nothing definite is known regarding its origin. Six guinea-pigs were inoculated intratesticularly with 1/5 slant of a young agar culture. The results are presented in table 6.

EXPERIMENTS WITH *B. ABORTUS* 14

The strain was a stock culture originally obtained from Dr. A. Eichhorn, who had isolated the culture from the uterine exudate of an artificially infected cow. Three guinea-pigs were inoculated intratesticularly with 1/5 slant of a young culture. Two animals (696 and 697) were repeatedly tested with "aborto"-protein, but no reactions were obtained. Both guinea-pigs gained in weight and when sacrificed

TABLE 6
EXPERIMENTS WITH B. ABORTUS 38, SERIES 1 *

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings
		Initial, Gm.	At Necropsy, Gm.				
679	1/5 slant	249	190	15th day, died	Abscess in right testis; general fibrinous peritonitis Typical abortion disease; spleen 2.4 gm.; foed in liver and kidneys	Testis and peritoneal fluid..... Spleen, liver, kidneys, urine, positive	Not tested B. abortus, 1:1000; B. melitensis 1:1,500
680	1/5 slant	269	353	74th day, positive skin reaction			
681	1/5 slant	245	190	Died, 7th day	Hemorrhagic orchitis, splenic tumor, toxemia (?) General lymphadenitis; spleen small (0.6 gm.); other tissues negative	Spleen positive; other organs not tested Spleen only tested and found sterile	Not tested Not tested
682	1/5 slant	266	248	Died, 54th day positive skin reaction			
683	1/5 slant	305	420	95th day, positive skin reaction	Spleen small (0.8 gm.); lymphnodes firm, but small; testes atrophic	Spleen \propto growth..... Testis, extensive growth.....	B. abortus 80, 1:800 Not tested
684	1/5 slant	395	267	19th day (moribund)			

* Two guinea-pigs—701 and 702—inoculated intratesticularly with an emulsion of the testis of guinea-pig 684 developed abortion disease with slight lesions, positive skin reaction, but negative spleen cultures.

on the 99th day presented normal and sterile organs. One guinea-pig (695) became infected. The history is given.

Guinea-Pig 695.—Weighing 460 gm., received intratesticularly 1/5 slant of *B. abortus* 14. Skin tests on the 44th day were positive. At necropsy (weight 292 gm.) on the 50th day, the spleen was small (0.7 gm.); the lymphnodes were soft and small. The right testis contained an abscess the size of a pea and the left epididymis exhibited several small abscesses. *B. abortus* was isolated from the pus of the abscesses, but not from the spleen, liver or urine. The serum agglutinated *B. abortus* 80 in a dilution of 1:2,000 and *B. melitensis* 11, 1:800.

EXPERIMENTS WITH *B. ABORTUS* 1

The culture was an old stock culture and had originally been obtained from Sir S. Stockman, who had isolated the strain from the uterine exudate of an artificially infected cow. Three guinea-pigs were inoculated intratesticularly with 1/5 slant of a young culture. Two animals (699 and 700) gave negative skin reactions and, when sacrificed on the 86th day, presented normal and sterile organs, while, according to the history given, one guinea-pig (698) became infected.

Guinea-Pig 698.—Weighing 565 gm., received intratesticularly 1/5 slant of *B. abortus* 1. Skin tests on the 44th and 66th day were positive. The guinea-pig was chloroformed on the 86th day after the inoculation, when it weighed 570 gm. The spleen was slightly enlarged (weight 1.3 gm.) and nodular; the liver showed a few scars, and the testes were atrophic. *B. abortus* was isolated from the kidneys, but not from the spleen, bonemarrow, liver, etc. The serum agglutinated *B. abortus* 80, 1:800.

These experiments indicate that intratesticular injections of *B. abortus* may produce an acute and fatal disease. Extreme emaciation and exhaustion combined with extensive inflammatory processes are probably the main causes of death. Every guinea-pig injected with strain 38 became infected, but the anatomic changes were only typical in one animal (680). Strain 80 acted similarly; one guinea-pig presented a small spleen when sacrificed on the 90th day, but showed changes in the ribs and testicles. Another animal gave negative skin tests repeatedly and was normal at necropsy on the 183rd day. Strains 1 and 14, probably old stock cultures, are less virulent than strains 80 and 38. The animals survived the injection, but only one guinea-pig of each series gave a positive skin reaction and presented distinct anatomic lesions. As far as the pathogenicity for guinea-pigs is concerned, the effect of these 2 strains cannot be distinguished from that of typical *B. melitensis* strains. In fact, the gross lesions, the cultural findings and the serologic results were indistinguishable from those previously noted in guinea-pigs inoculated with *B. melitensis* strains 11

or 27. Subcutaneous injections of strain 80 gave positive infections, but in only 2 animals was the pathologic picture typical.

The differences in pathogenicity cannot be ascribed to the amounts of culture injected. Young cultures were suspended in saline and the turbidity of the emulsion was standardized against a typhoid vaccine containing 1 billion bacteria per c.c. One tenth or 1/5 of an agar slant of *B. abortus* contained about 1,000-2,000 million viable organisms and was approximately the same for each of the 4 strains. It is naturally impossible to make conclusive statements with regard to the virulence of a *B. abortus* strain until further knowledge relative to the individual susceptibility of the guinea-pigs has been accumulated, but the few carefully controlled observations rather forcibly suggest that old stock cultures are not only less toxic, but also less invasive than recently isolated strains or infected material derived from bovine, porcine oravian sources. This contention is supported by some tests conducted with a strain of *B. abortus* isolated from a case of swine abortion.

INTRAPERITONEAL INJECTIONS OF *B. ABORTUS* CULTURES OF PORCINE ORIGIN

In 1920, Dr. J. Traum furnished the writers with a culture of *B. abortus* isolated from the liver of an aborted swine fetus. The strain grew profusely, in contrast with the bovine *B. abortus* strains, but could not be distinguished biochemically or serologically from the ordinary stock cultures kept in this laboratory. However, the porcine *B. abortus* manifested noteworthy invasive properties for monkeys. These facts have already been noted in papers I, II and III of this series. Twenty-four guinea-pigs were inoculated with 1/20 slant of a vigorously growing agar culture of the second generation.

The clinical and necropsy findings are summarized in table 7.

The data presented in table 7 proved the highly virulent character of a porcine strain of *B. abortus*. Six animals succumbed to the infection in from 17-40 days after the intraperitoneal injection. The loss in weight varied from 73.5 to 345 gm. and only 6 of the 24 animals showed a gain of 10 to 195 gm., or an average increase of 41 gm. The strain exhibited pyogenic properties exemplified by abscess formation in the peritoneal cavity, omentum and lymphnodes, likewise in some instances in the spleen and liver. The testicles, epididymis and seminal vesicles of all the animals used in the test were destroyed by suppurative processes. The spleen was usually enlarged, but 2 animals presented, at least macroscopically, a normal splenic capsule and pulp. Nine

animals had involvements of the ribs and long bones, while 11 showed lesions only in the radiocarpal and tibiotarsal regions. The lungs and kidneys were never diseased. *B. abortus* was readily isolated from the spleen, testicles, etc., and the blood serum agglutinated the representatives of the *Brucella* group in dilution of 1:800-1:2,000.

Constant infections with definite localization of the lesions in the sex organs, bone structures, spleen and lymphnodes were not observed with an inoculation of less than 2,000 million organisms. Guinea-pigs purposely procured from several breeders were employed, and the striking results could therefore only be attributed to the inherent properties

TABLE 7
NECROPSY FINDINGS ON GUINEA-PIGS INOCULATED WITH *B. ABORTUS* CULTURES OF
PORCINE ORIGIN

Died or Sacrificed	Number	Average Loss or Gain in Weight, Gm.	Spleen, Average Weight	Lymph-nodes	Liver Abscesses	Abscess in Omentum	Abscess in Testes or Epididymis	Involvement of Ribs	Radiocarpal Tibiotarsal Region
Died, 17 days	2	167	1.3	Abscesses 2	0	2	1 (1 female)	0	0
Died, 18 days	1	112	0.8	Enlarged	0	1	1	1	0
Died, 20 days	1	196	0.9	Small	0	1	1	1	0
								both sides	
Died, 38-40 days	3	126	1.1	3 enlarged	1	3	2 (1 female)	2	1
Sacrificed 55 days	2	345	3.35	2 enlarged	1	2	1 (1 female)	0	0
Sacrificed 69 days	14	8-73.5 6+41.0	3.57	12 enlarged	9	8	4 (10 females neg.)	5	9
Sacrificed, 134 days	1	-238	2.21	1 enlarged	1	1	1	0	1
	24	21 enlarged	12	18	11	9	11

of the strain and not to the susceptibility of certain animals. Furthermore, F. Hayes and J. Traum²⁴ and Schroeder²⁵ have recently reported identical results, using the same and other porcine strains on guinea-pigs obtained from different sources.

INOCULATIONS OF GUINEA-PIGS WITH INFECTED MILK, GUINEA-PIG TISSUES AND CULTURES OF *B. ABORTUS*

Since 1915, the writers have been interested in the occurrence of *B. abortus* in "certified milk" and later in the problem of cutaneous hypersensitiveness in guinea-pigs infected with *B. abortus* or *B. melitensis*. In the course of these studies, several hundred male and female

²⁴ Jour. Amer. Vet. Med. Assn., 1922, 60, p. 435, and personal communication.

²⁵ Jour. Amer. Vet. Med. Assn., 1922, 60, p. 560.

guinea-pigs were inoculated with milk sediment, spleen emulsions of infected animals or cultures of *B. abortus*, etc. The necropsy examinations of 114 animals were conducted according to the principles laid down in the paragraph on methods, and the data, although originally collected for entirely different purposes, can therefore be used in a discussion dealing with the pathogenicity of *B. abortus* for guinea-pigs. For the sake of brevity results of importance are presented in tables 8 and 9.

It is to be regretted that the data summarized in tables 8 and 9 do not lend themselves to a discussion of the relative susceptibility of guinea-pigs to *B. abortus*, nor do the findings permit any conclusions relative to the virulence of the various strains employed. In the first place, it was obviously impossible to determine the number of viable organisms in the injected tissues or milk, and, secondly, in those instances in which the number of inoculated bacteria was known, namely, not more than 1,000-2,000 million bacteria per dose, it was, for various other reasons, necessary to keep the animals under observation for at least 300-400 days. As early as 1916, it was noted that a series of 48 guinea-pigs injected with old stock cultures isolated in 1910 and 1911 not only survived the infection, but 8 animals failed to give skin reactions and on necropsy were found to be free macroscopically, microscopically and culturally from *B. abortus*. At that time serum tests were not conducted systematically, and this series can therefore be considered only from the standpoint of gross lesions. The data in tables 8 and 9 have been arranged according to the material which was used for the inoculations. It is quite evident that from the tables it is possible to classify the infections as acute, subacute or chronic. The following facts are apparent:

Guinea-pigs inoculated with infected milk, cultures, etc., and permitted to live for from 29-200 days always exhibited enlarged, firm lymphnodes; the spleen was typical and enlarged (1.2 to 10.5 gm. or an average of 4.69 gm.) in 80% of the cases. Normally sized spleens were more frequently seen among females than males. Animals, which lived from 200-395 days, classified here as chronic infections, had small lymphnodes and normal spleens. Sixteen of 17 males, which came to necropsy on the 300-389th day, presented spleens of an average of 0.83 gm. In many instances, however, this organ was adherent to the abdominal wall and, on section, it was strikingly fibrous and dry. Atrophy of the testicles was always present, while abscess formation in these tissues was found in 12, or 70%, of the animals. In the acute

TABLE 8
B. ABORTUS INFECTIONS IN GUINEA-PIGS
Male and Female Guinea-Pigs Inoculated with Milk Sediment or Spleen Emulsions of Infected Guinea-Pigs

Day on Which Sacrificed	Num- ber of Guinea- Pig		Lymphnodes				Spleen				Liver		Lungs		Kidneys		Testicles		Uterus		Bone Lesions				Cultures from				Agglutination Titer of Serum																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
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TABLE 9
CHRONIC B. ABORTUS INFECTIONS IN GUINEA-PIGS
Male and Female Guinea-Pigs Inoculated with B. Abortus Cultures

[illegible]

infections, a bilateral atrophy of the sex organs was noted in 60% and definite abscesses in 14%. Liver and lung lesions occurred more often in the course of the subacute disease; in fact, no pathologic processes in these organs were recorded in animals inoculated with cultures. Kidney lesions were rare, and involvement of the bones of the extremities was somewhat more frequently noted in the chronic than in the acute or subacute cases. Blindness was seen in 2 males suffering from a chronic infection. Isolation of *B. abortus* from the spleen was rarely successful in the chronic infections, but the pus of the focal lesions invariably contained a pure growth of the organism. The serum reactions rarely indicated the age of the disease; numerous guinea-pigs tested on the 100-150th day after inoculation agglutinated *B. abortus* in dilutions 1:200-1:400, while others living for more than 300 days clumped the organisms in dilutions as high as 1:4,000.

SUMMARY

The course and the character of the lesions produced in 160 guinea-pigs by the inoculation of 1,000-2,000 million organisms of different strains of *B. abortus* or body fluids or tissues containing this bacterium can be summarized in 3 groups, namely:

(a) Acute toxic and fatal infections frequently follow the intratesticular inoculation of certain stock cultures. In this series, they occurred also following the intraperitoneal injection of a strain of *B. abortus* isolated from an aborted swine fetus. Lesions in the spleen are slight or absent; *B. abortus* can be found in all tissues, but not in the heart blood.

(b) The classical subacute infections, as described by T. Smith and Fabyean, with very low mortality, pronounced cutaneous hypersensitiveness, positive serum reactions and distinct lesions in the lymphnodes, spleen, liver, kidneys, lungs, bones, etc., follow quite regularly the subcutaneous, intraperitoneal or intratesticular inoculation of infected material or recently isolated cultures. Old laboratory strains may occasionally produce this form of the disease in a few guinea-pigs. The spleen is always enlarged and nodular, while the other tissues may or may not be affected. Some strains exhibit a tendency to localize in the bone structures.

(c) Chronic infections with cutaneous hypersensitiveness, marked positive serum reactions and small spleens, but definite lesions in the genital organs or bones of the extremities, are noted in guinea-pigs injected with old stock cultures. Male guinea-pigs present at necropsy

a small spleen, abscesses in the testicles, epididymis, or a suppurative seminal vesiculitis and swelling of the radiocarpal or tibiotarsal regions. Cultures can frequently be procured only from the pus of these lesions. Females may fail to show gross changes; they are never pregnant, and they may present a vaginal discharge. The spleen is always fibrous and atrophic, but may occasionally yield a positive culture. The existence of an infection can only be established by skin tests with aborto protein, by serum agglutination tests, or by careful histologic studies.

The average weight of the spleen in the various groups is as follows:

	Average Body Weight	Spleen Weight
Normal guinea-pigs (50).....	485.4 gm.	0.79 gm.
Group A—Acute and fatal (12).....	376.4 gm.	1.3 gm.
Group B—Subacute (10)	440.0 gm.	4.69 gm.
Group C—Chronic (14)	607.5 gm.	0.83 gm.
<i>B. melitensis</i> infections (17).....	532.0 gm.	1.70 gm.

The experiments reported in these paragraphs support the view that *B. abortus* may produce in guinea-pigs an inoculation disease with tissue injury of varying degree of intensity. This conclusion applies in particular to the changes in the spleen and to the infections produced with cultures. The acute infection, as a rule, extends over a relatively short period and reparative processes appear at the end of a month. These animals may recover but harbor the inoculated *B. abortus* in foci of the genital organs. This sexotropism, which follows any form of injection, is remarkably constant and is of considerable diagnostic value. Infections involving all the tissues of the body with the exception of the muscles follow the injection of a porcine strain of *B. abortus*. The enlargement of the spleen has been most striking and constant in guinea-pigs injected with body fluids or tissues infected with bovine *B. abortus*.

The observations are not sufficiently extensive to warrant final conclusions, but they indicate that old *B. abortus* cultures may either fail to cause a disease, or the anatomic lesions are so slight that they cannot be seen with the unaided eye. It is this group of cases which resembles anatomically the infections caused by the intratesticular or intraperitoneal injections of the majority of *B. melitensis* cultures.

DISCUSSION

This comparative study has clearly shown that the two organisms of the *Brucella* group are pathogenic for guinea-pigs, but *B. abortus* is,

as a rule, slightly more invasive and virulent than *B. melitensis*. As has been pointed out, this statement deserves further experimental study with *B. melitensis* strains derived from caprine or human sources. In paper III of this series of investigations, it has been shown that *B. melitensis* is far more invasive for monkeys than *B. abortus*, while the tests on guinea-pigs apparently prove the reverse. The organisms which have been tentatively placed, on account of their bacteriologic similarity, into one group, can probably in the light of the studies of this laboratory be distinguished by their serologic behavior and pathogenicity for monkeys and guinea-pigs. For the experimental pathologist this peculiar variability in the pathogenicity for laboratory animals offers many fascinating problems. The solution of the question "Can the *B. abortus* be transformed into the *B. melitensis* and vice versa?" deserves careful attention in the future. Some attempts along this line have been made without obtaining any definite leads. Furthermore, cross-immunization tests have been undertaken with equally negative results. In two series, 5 guinea-pigs were treated for several months with ascending doses of fresh heat killed cultures of *B. melitensis* 11 and 20, *B. paramelitensis* 9 and *B. abortus* 80. Ten days after the last injection, each animal, together with 5 control, normal guinea-pigs, were inoculated subcutaneously with 2,000 million *B. abortus* strain 80, or strain swine fetus. Certain normal and certain immunized animals died acutely following the injection of the porcine *B. abortus* strain, while the surviving guinea-pigs, when sacrificed on the 60th-100th day after the test inoculation, presented either moderately severe lesions when injected with *B. abortus* 80 or extensive tissue changes when tested with the highly invasive porcine strain. The vaccinated guinea-pigs exhibited the same lesions as the control animals. Studies are in progress to solve the same problems on rats and mice.

The anatomic lesions produced in guinea-pigs by the injection of *B. melitensis* deserve some consideration and may perhaps explain some of the necropsy findings reported in human and caprine cases of undulant fever. As the mortality from this disease is usually low, the number of carefully conducted human postmortems is relatively small, and the available information is correspondingly small and incomplete. However, some facts immediately attract attention: Thus, it is stated by Kennedy²⁶ that the lymphnodes are predominantly diseased, the spleen is enlarged and, according to Basset-Smith,²⁷ the latter organ

²⁶ Report Mediterranean Fever Commission, 1906, Part IV, p. 94.

²⁷ Jour. Roy. Army Med. Corps. 1908, 10, p. 70.

shows microscopically a marked increase in endothelial cells and a decrease in lymphatic tissues. Not only the mesenteric lymphnodes, but also the axillary and inguinal nodes are enlarged and contain innumerable specific organisms.

Fluctuating swellings over the sternum and ribs have sometimes been mistaken for abscesses, but no pus could be procured. In reading these descriptions, one recalls the rib lesions noted in guinea-pigs. The clinical histories of Luger,²⁸ Lombard and Bégner²⁹ and others mention epididymitis, orchitis, periostitis and mono-arthritis as meta-static processes, which are not uncommon in the course of an undulant fever attack. Eyre³⁰ records amenorrhea and occasionally abortion as sequelae of *B. melitensis* infections in women.

The Reports of the Mediterranean Commission, of Mohler and Hart³¹ and of others dealing with the necropsy findings of goats infected with *B. melitensis* record an enlarged spleen, general enlargement of the lymphnodes, distinct hyperemia of the liver and kidneys. Abortion is a frequent sequel of Malta fever in goats. Furthermore, *B. melitensis* has been isolated regularly from the spleen, lymphnodes, mammary glands and occasionally from the urine. The diseased tissues have not been studied extensively. Spagnolio,³² who examined the spleens of 42 goats, noted focal nodules consisting of epithelial cells and central necroses, while Neri³³ reported the lesions of a definite mastitis. There is no doubt that *B. melitensis* possesses in man and goats, just as *B. abortus* in guinea-pigs, a predilection for the perivascular and subcapsular tissues of the spleen, lymphnodes and bone structures. The specific ubertropism of both organisms is only an expression of a marked sexotropism, which in females may lead to abortion or localization of the bacterium in the udder; while in males it manifests itself in the form of suppurative processes in the testicles, epididymis, etc. The latter fact, known for *B. melitensis* in man, has recently been recognized by Buck, Creech and Ladson³⁴ for *B. abortus* in bulls. It is evident from the observations recorded in this paper and the theoretical considerations just presented that the bacteria belonging to the *Brucella* group exert their specific pathogenic properties

²⁸ Deutsch. med. Wchnschr., 1921, 47, p. 321.

²⁹ Presse Méd., 1921, 29, p. 753.

³⁰ Handbuch d. path. Mikroorg., 1912, p. 438.

³¹ Twenty-Fifth Annual Report of the Bureau of Animal Industry, 1908, p. 279.

³² Centralbl. f. Bakteriöl., Ref., 1908, 42, p. 677.

³³ Ann. d'ig. sper., 1912, 21, p. 321.

³⁴ Jour. Agric. Res., 1919, 17, p. 239.

not only in the original host, the goat, the cow and the swine, but also in the small laboratory rodents. It is therefore not unlikely that our knowledge concerning the pathogenesis and immunity, which is intimately connected with the control and eradication of two important economic diseases, namely, infectious abortion in cattle and swine and undulant fever in goats, can probably be advanced by a careful study of the inoculation disease in guinea-pigs.

CONCLUSIONS

The experiences collected in this paper indicate that certain strains of *B. melitensis* are capable of producing an acute, subacute or chronic inoculation disease in guinea-pigs. The gross anatomic and the histologic changes resemble those commonly noted in guinea-pigs infected with *B. abortus*. In fact, it is sometimes impossible to distinguish the two infections without careful serologic cross absorption tests. Four cultures, 2 old strains isolated from human cases of undulant fever in 1896 and 1904, one human strain isolated in 1915, and one caprine strain recovered from goat's milk in 1917, infected guinea-pigs regularly, while 18 other strains proved either nonpathogenic or produced in exceptional instances lesions in the spleen and lymphnodes. Aside from the inherent pathogenic property of certain strains, it is not unlikely that the individual susceptibility of the guinea-pigs and the mode of injection are in a large degree responsible for the course and the character of the infection. Intratesticular injections have been used most frequently.

Thirty-four of 44 guinea-pigs, which were sacrificed 44-155 days after the injection with cultures, presented advanced tuberculosis-like lesions in the spleen and lymphnodes. The average weight of the diseased spleen was 1.7 gm. The largest spleen observed in these experiments weighed 3.5 gm. Localization of the disease in the liver and testicles, as well as proliferation of the bone structure, has been recorded. As a whole, the lesions produced by *B. melitensis* in guinea-pigs are less extensive and severe than those produced by *B. abortus*.

Experiments extending over several years support the well known fact that *B. abortus* may produce in guinea-pigs an interesting inoculation disease. However, the degree of tissue injury in the spleen and lymphnodes may vary considerably. Sexotropism, which follows any form of inoculation whether caused by infected material or cultures, is remarkably constant. Infections involving all the tissues of the body,

with the exception of the muscles, has been characteristic for a strain of *B. abortus* isolated from an aborted swine fetus. Enlargement of the spleen with a maximum weight of 10.5 gm. and an average weight of 4.69 gm. has been noted in guinea-pigs injected with infected milk or tissues. Old laboratory cultures, as a rule, produce lesions which are so slight that they cannot be seen with the unaided eye. This group of cases resembles anatomically the infections caused by intratesticular or intraperitoneal injections of certain *B. melitensis* cultures.

Recently isolated strains and, in particular, cultures or milk specimens procured from goats affected with malta fever should be tested for pathogenic properties on guinea-pigs.